OTME-16. POLIO VIROTHERAPY OF MURINE BRAIN TUMORS CAUSES MICROGLIA/MACROPHAGE PROLIFERATION AND INFLAMMATION THAT IS POTENTIATED BY IMMUNE CHECKPOINT BLOCKADE

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PVSRIPO is a novel viral immunotherapy that has shown evidence of efficacy in a phase I clinical trial for recurrent GBM, resulting in 21% survival rate at 36 months following treatment. To improve clinical response rate, it is critical to resolve the mechanisms of action and therapy resistance *in vivo*, thereby designing effective combination therapy strategies. We used immunocompetent mouse models of glioma (CT2A) and metastatic melanoma (B16) to dissect early and late events following virotherapy with PVSRIPO. A blinded systematic review of the pathology from 62 intracranial tumors, collected on different days following PVSRIPO (or control) treatment, was performed. An overall treatment effect, measured by tumor shrinkage, dis-cohesive growth pattern, microglia enrichment, was present in 88% of tumors on day 8, but the tissue response rate fell to 42% on days 10 & 12, and 14% on day 15. The control group showed no treatment effect throughout. RNAseq from the same set of samples showed acute induction of type-I interferon-related inflammation that faded with time in Gene Set Enrichment Analysis. This suggests that sustaining adaptive antitumor immunity elicited by immediate intratumor type-I IFN-dominant inflammation is critical to long term remission. Careful review of the post treatment pathology revealed an early enrichment of both T cells and microglia in the tumor microenvironment with a high Ki-67 proliferation index. We propose that the PVSRIPO therapy effect is dependent on macrophage/microglia mediated cellular immune response, likely in response to direct viral infection. This suggests potential therapeutic interventions, including blockade of the PD1:PD-L1 immune checkpoint, to potentiate antitumor CD8+T cells in response to PVSRIPO therapy. Indeed, combination therapy with αPD-L1 antibody in the CT2A model showed higher long term remission (37%, n=11), compared to either monotherapy; this effect is CD8+T cell- and macrophage-dependent, demonstrated by depletion studies *in vivo*.

OTME-17. SINGLE CELL CHARACTERIZATION OF THE IMMUNE MICROENVIRONMENT OF MELANOMA BRAIN AND LEPTOMENINGEAL METASTASES

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Melanoma brain metastases (MBM) and leptomeningeal metastases (LMM) are two manifestations of melanoma dissemination to the CNS with vastly different survival outcomes. Analysis of single cell RNA-Seq data from 43 clinical specimens has uncovered a distinct, immune-suppressed T cell landscape in the LMM microenvironment that is distinct to those of the brain and skin metastases. An LMM patient with an extraordinarily long survival and documented response to therapy demonstrated an immune repertoire that was distinct from those of typical poor survivors and more similar to CSF from non-LMM donors. Analysis of serial specimens over the course of therapy demonstrated reductions in melanoma cells and macrophages, coupled with increased levels of T cells and dendritic cells in the CSF of the extraordinary responder, whereas poor survivors showed no improvement in T cell responses. In MBM patients, targeted therapy and immunotherapy was associated with increased immune infiltrate, with similar T cell transcriptional diversity noted between skin metastases and MBM - suggestive of immune cell trafficking into the brain. Treatment with targeted therapy was associated with an enrichment of CD8 T cells. Immunotherapy was associated with a more diverse lymphocyte landscape and higher numbers of antibody-producing cells. These findings were confirmed by multiplexed staining of patient specimens and using an immune-competent mouse model of MBM. Correlation analysis across the entire immune landscape identified the presence of a rare, novel population of dendritic cells (DC3s) to be correlated with increased overall survival, regardless of disease site/treatment. The presence of DC3s positively regulated the immune environment of both patient samples and preclinical melanoma models through modulation of activated T cells and MHC expression in the tumor. Our study provides the first comprehensive atlas of two distinct sites of melanoma CNS metastases and identifies rare populations of cells that underlie the biology of this devastating disease.

OTME-18. TARGETED CRISPR/CAS9 GENE-EDITING REGULATES THE BRAIN TUMOR ENVIRONMENT

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Glioblastoma multiform (GBM) is the most common malignant brain tumor. Recent immunotherapy has demonstrated potential to treat GBM. However, the immune suppressive tumor environment in the brain represents a significant barrier for the treatment of GBM. Overexpression of programmed death ligand-1 (PD-L1) in GBM tumor cells and macrophages plays a key role in GBM vitality, proliferation, and migration, while also suppressing the immune system. We developed a CRISPR/Cas9 gene-editing system to delete whole cell PD-L1. Human PD-L1 targeted sgRNA were cloned into CRISPR/Cas9 plasmids with or without an HDR templet. CRISPR/Cas9 were treated to human GBM U87 cells for 15, 30, 60, 120 and 240 minutes. The intracellular concentration of CRISPR/Cas9 exhibited a time-dependent increases. A GFP tagged CRISPR/Cas9 plasmid was developed to test the transfection efficacy. Higher levels of GFP+ U87 cells were observed at day 3. CRISPR/Cas9 showed a greater PD-L1 knockout at day 3. The PD-L1 reduction limited the proliferation of U87 cells. A scratch assay showed that PD-L1 deletion inhibited the migration of U87 cells. An in vitro GBM model was developed by co-cultivation of U87 cells and macrophages. CRISPR/Cas9 treated co-cultures changed the ratios of U87 cells and macrophages and polarized tumor associated macrophages (TAM) from M2 toward M1. CRISPR/Cas9 gene-editing effectively deleted PD-L1 in U87 cells. Successful deletion of PD-L1 prevented U87 cells growth and migration, and altered the TAMs plasticity and the tumor environment.

OTME-19. REGULA REGULATION OF GLIOMAGENESIS AND STEMNESS THROUGH ACID SENSOR ASIC1A

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Glioblastoma multiforme (GBM) is the most prevalent and aggressive type of adult gliomas. Despite intensive therapy including surgery, radiation, and chemotherapy, invariable tumor recurrence occurs, which suggests that glioblastoma stem cells (GSC) render these tumors persistent. Recently, GSC differentiation has emerged as an alternative method to treat GBM, and most of current studies aim to convert GSC to neurons by a combination of transcriptional factors. As the tumor microenvironment is typically acidic due to increased glycolysis in tumor cells, here, we explored the role of acid-sensing ion channel 1a (ASIC1a), an acid sensor, as a tumor suppressor in gliomagenesis and stemness. The bioinformatics data from TCGA shows that ASIC1 expression levels in GBM tumor tissues were lower than those in normal brain, and glioma patients with elevated ASIC1 expression have longer survival than those with lowered ASIC1 expression. Our immunohistochemistry data from tissue microarray shows that ASIC1a expression is negatively correlated with glioma grading. Functional studies reveal that the downregulation of ASIC1a promotes glioma cell proliferation and invasion, while upregulation of ASIC1a inhibits their proliferation and invasion. Furthermore, ASIC1a suppresses glioma cells' growth and proliferation through G1/S arrest and apoptosis induction. Mechanistically, ASIC1a negatively modulates glioma stemness via inhibition of the Notch signaling pathway and GSC markers CD133 and ALDH1. Our findings indicate that ASIC1a is a tumor suppressor in gliomagenesis and stemness and may serve as a promising prognostic biomarker and target for GBM patients.

OTME-20. CHITINASE-3-LIKE-1(CHI3L1) PROTEIN COMPLEXES REGULATE THE IMMUNOSUPPRESSIVE MICROENVIRONMENT IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and highly malignant brain tumor in adults. Despite advances in multimodal treatment, GBM re-

mains largely incurable. While immunotherapies have been highly effective in some types of cancer, the disappointing results from clinical trials for GBM immunotherapy represent continued challenges. GBM is highly immunosuppressive and resistant to immunotherapy because of glioma cells escaping from immune surveillance by reprograming the tumor microenvironment (TME). However, understanding the mechanisms of immune evasion by GBM remains elusive. Based on unbiased approaches, we found that Chitinase-3-like-1 (CHI3L1), also known as human homolog YKL-40, is highly expressed in GBM, which is regulated by the CHI3L1-PI3K/AKT/mTOR signaling in a positive feedback loop. Gain- and loss-function studies reveal that CHI3L1 plays a predominant role in regulating an immunosuppressive microenvironment by reprogramming tumor-associated macrophages (TAMs). Using the liquid chromatography-mass spectrometry and orthogonal structure-based screening, we found that Galectin-3 binding protein (Gal3BP) and its binding partner, Galectin-3 (Gal3), can interact competitively with the same binding motif on CHI3L1, leading to selective migration of M2-like versus M1-like bone marrow-derived macrophages (BMDMs) and resident microglia (MG). Mechanistically, the CHI3L1-Gal3 protein complex governs a transcriptional program of NFκB/CEBPβ to control the protumor phenotype of BMDMs, leading to inhibition of T cell infiltration and activation in the GBM TME. However, Gal3BP can reverse CHI3L1-Gal3 induced signaling pathway activation and subsequent protumor phenotype in TAMs. Based on protein binding motifs, a newly developed Gal3BP mimetic peptide can attenuate immune suppression and tumor progression in the syngeneic GBM mouse models, including decreasing M2-like TAMs and increasing M1-like TAMs and T cell infiltration. Together, these results shed light on the role of CHI3L1 protein complexes in immune evasion by glioblastoma and as a potential immunotherapeutic target for this devastating disease.

OTME-21. THE ROLE OF GLIOBLASTOMA ASSOCIATED MESENCHYMAL STEM CELLS IN IMMUNE SUPPRESSION

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Glioblastoma (GBM) is an aggressive brain cancer, with an overall survival of 14.6 months. The tumor microenvironment in GBM plays major roles in immunosuppression and modulation of the response to therapies. GBM patients with higher levels of mesenchymal stem like cells (G-MSC) show poor overall survival as compared to patients with no/lower G-MSC levels. Our lab found that levels of G-MSC corelate with CD4+ T cells in humans and murine models of GBM, and with immunosuppressive molecules like PTGS2, the gene for cyclooxygenase 2. To investigate the mechanism by which G-MSCs promote immunosuppression, we isolated G-MSCs from an orthotopic mouse model of GBM and subjected them to RNASeq analysis to obtain an unbiased picture of transcriptomic changes occurring upon activation. We identified changes in multiple immune modulating pathways involving antigen presentation, leukocyte migration and activation, and immune checkpoints. Our findings indicate that G-MSCs represent a key immune modulating faction in the microenvironment. Further dissection of the role of these cells in immune modulation will aid us in understanding the biology of the brain tumor microenvironment and identifying potential combination therapies.

OTME-22. BIOINFORMATIC EVALUATION OF ECM MOLECULES AND ANGIOGENIC ASSOCIATE GENES IN DIFFUSE MIDLINE GLIOMA (DMG): MAPPING THE TUMOUR MICROENVIRONMENT

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Paediatric Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating cancer of an extremely aggressive nature, located in the pontine area of the brain. DIPG primarily affects children, with the average age of diagnosis between 6 and 7 years. Unfortunately, the outlook and overall survival remains bleak. While there has been impressive progress in identifying genes that are central to and drive DIPG growth; there remains several gaps in documenting the DIPG microenvironment landscape. The focus of this study is to begin to examine mRNA expression of genes associated with blood vessel development, angiogenesis, and extracellular matrix molecules (ECM) in normal brain development and DIPG by utilizing publicly available genomic datasets. In-depth bioinformatics from GSE26576 dataset included differential expression and gene ontology (GO) with KEGG pathway analyses using Gene Expression Omnibus (GEO) and DAVID, which have revealed a number of significantly upregulated genes that may affect DIPG angiogenic processes ($p<0.05$). 38 of such genes from 9 different GO terms were then included in a protein-to-protein interaction network that revealed a surprising connection between *MMP16, CSPG4* and *COL11A1*. Subsequently, using R2 genomic visualisation platform from publicly available single cell RNAseq data we showcased the difference in their individual expression based on the molecular subtypes of DIPG histone 3 (H3) mutation (K27M, wild type and G34R) with a strong statistical significance ($p<0.05$). Interestingly, during normal paediatric development such genes showed consistent expression, suggesting their potential complications in DIPG angiogenesis. Overall, this bioinformatic approach has led to the identification of a set of interacting genes that will inform our *in vitro* and *in vivo* studies. This information will add to the documentation of the host/tumour microenvironment landscape and our plan is to continue to explore this area to map the spatial and temporal expression of these genes.

OTME-23. SINGLE-CELL TRANSCRIPTOMIC AND EPIGENOMIC IMMUNE LANDSCAPE OF ISOCITRATE DEHYDROGENASE STRATIFIED HUMAN GLIOMAS

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The brain tumor immune microenvironment (TIME) continuously evolves during glioma progression, but a comprehensive characterization of the glioma-centric immune cell repertoire beyond a priori cell states is uncharted. In this study, we performed single-cell RNA-sequencing (scRNA-seq) and single cell- Assay for Transposase-Accessible Chromatin using sequencing (sc-ATAC-seq) on ~100,000 tumor-associated immune cells from seventeen isocitrate dehydrogenase (IDH) mutation classified primary and recurrent human gliomas and non-glioma brains (NGBs). Our analyses revealed sixty-two transcriptionally distinct myeloid and lymphoid cell states within and across glioma subtypes and we noted microglial attrition with increasing disease severity concomitant with invading monocyte-derived cells and lymphocytes. Specifically, certain microglial and monocyte-derived subpopulations were associated with antigen presentation gene modules, akin to cross-presenting dendritic cells (DCs). We identified cytotoxic T cells with poly-functional cytolytic states mostly in recurrent IDH-wt gliomas. Furthermore, ligand-receptor interactome analyses showed a preponderance of antigen presentation and phagocytosis over the checkpoint axis in IDH-wt compared to IDH-mut gliomas. Additionally, our sc-ATAC-seq analyses revealed differences in regulatory networks in NGBs, IDH-mut and IDH-wt glioma associated immune cells. In particular, we noted abundant usage of inflammatory transcription factors (TFs) as exemplified by Nuclear factor kappa B and Activator Protein-1 TF family in IDH-wt microglia when compared with microglia from IDH-mut and NGBs. Unique features such as amplification of 11- Zinc Finger Protein accessibility were restricted to monocyte derived cells and were not observed in microglia. Finally, sc-ATAC-seq profiles of $CD8^+$ exhausted T cells from IDH-wt showed strong enhancer accessibility on Cytotoxic T-lymphocyte-associated protein 4, Layilin and Hepatitis A Virus Cellular Receptor 2 but no enrichment on PDCD1 (gene encoding Programmed cell death protein 1) was seen. In summary, our study provides unprecedented granular detail of transcriptionally defined glioma- specific immune contexture that can be exploited for immunotherapy applications.

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